Developmental Cell
Article

Control of Plant Organ Size by *KLUH/CYP78A5*-Dependent Intercellular Signaling

Elena Anastasiou,[1] Sabine Kenz,[1] Moritz Gerstung,[2] Daniel MacLean,[3] Jens Timmer,[2] Christian Fleck,[2] and Michael Lenhard[1,4,*]
[1]Institut für Biologie III, Universität Freiburg, Schänzlestrasse 1, D-79104 Freiburg, Germany
[2]Institut für Physik, Universität Freiburg, Hermann-Herder-Strasse 3a, D-79104 Freiburg, Germany
[3]The Sainsbury Laboratory
[4]John Innes Centre
Colney Lane, Norwich NR4 7UH, UK
*Correspondence: michael.lenhard@bbsrc.ac.uk
DOI 10.1016/j.devcel.2007.10.001

SUMMARY

Plant organs grow to characteristic sizes that are genetically controlled. In animals, signaling by mobile growth factors is thought to be an effective mechanism for measuring primordium size, yet how plants gauge organ size is unclear. Here, we identify the *Arabidopsis* cytochrome P450 *KLUH* (*KLU*)/*CYP78A5* as a stimulator of plant organ growth. While *klu* loss-of-function mutants form smaller organs because of a premature arrest of cell proliferation, *KLU* overexpression leads to larger organs with more cells. *KLU* promotes organ growth in a non-cell-autonomous manner, yet it does not appear to modulate the levels of known phytohormones. We therefore propose that KLU is involved in generating a mobile growth signal distinct from the classical phytohormones. The expression dynamics of *KLU* suggest a model of how the arrest of cell proliferation is coupled to the attainment of a certain primordium size, implying a common principle of size measurement in plants and animals.

INTRODUCTION

Growth of plant organs up to their species-specific size is controlled by the developmental genetic program (Anastasiou and Lenhard, 2007; Ingram and Waites, 2006). It occurs in two successive, linked phases: in the first phase, cells in the organ primordium produce cytoplasmic mass and divide. Once sufficient cells have been generated, cell proliferation ceases and the postmitotic cells expand. Cell expansion is often, but not always, accompanied by ploidy increase due to endoreduplication. These two processes will be referred to as growth by proliferation and growth by expansion, respectively.

Several factors that control either of the two processes have been isolated. The AP2-domain transcription factor AINTEGUMENTA (ANT) promotes organ growth by maintaining the competence of cells to proliferate (Mizukami and Fischer, 2000). *ANT* activity is stimulated by ARGOS, a protein of unknown function acting downstream of auxin signaling (Hu et al., 2003). Similar to *ant* mutants, plants lacking the presumed transcription factors JAGGED (JAG) and NUBBIN (NUB) show a premature arrest of cell proliferation in lateral organs, leading to a characteristic jagged organ shape (Dinneny et al., 2004, 2006; Ohno et al., 2004). Cell proliferation is also reduced when the putative transcriptional coactivator ANGUSTIFOLIA3/GRF-INTERACTING FACTOR1 (AN3/GIF1) is mutated, leading to smaller, narrower leaves and flowers (Horiguchi et al., 2005; Kim and Kende, 2004). Cell proliferation in leaves, particularly at the margins, is restricted by the TCP protein CINCINNATA in *Antirrhinum* and by its orthologs in *Arabidopsis* (Nath et al., 2003; Palatnik et al., 2003). In contrast to these TCP genes, which appear to act on most leaf cell types, the redundant *PEAPOD1* and *PEAPOD2* genes limit the proliferation of a subpopulation of leaf cells including stomatal and vascular precursors (White, 2006). The duration of cell proliferation is also restricted by the E3 ubiquitin ligase *BIG BROTHER* (*BB*), mainly in floral organs and the stem (Disch et al., 2006). A unifying theme to emerge from these studies is that most of the factors isolated to date seem to influence the timing, rather than the rate, of proliferation, suggesting that the transition from the proliferation to the expansion phase is a crucial point of regulation in organ-size control. Subsequently, growth by expansion is promoted by *ARGOS-LIKE1*, acting downstream of brassinosteroids (Hu et al., 2006), and by the cytochrome P450 *ROTUNDIFOLIA3* (*ROT3*), which is involved in the biosynthesis of brassinosteroids and stimulates expansion, specifically in the longitudinal direction (Kim et al., 2005). In petals, the activity of these genes is antagonized by the putative transcription factor *BIGPETAL*, which limits cell expansion to restrict petal size (Szecsi et al., 2006). Endoreduplication is a prerequisite for the massive cell expansion observed, for example, in leaves, and mutations that block endoreduplication cause dwarfing due to reduced cell expansion (Sugimoto-Shirasu et al., 2005).

Despite the recent progress in identifying factors involved in size control in plants, two key questions remain unanswered. What is being measured by the cells in a developing organ primordium to allow them to make a decision about further growth? And, how is this decision coordinated across the primordium? A possible answer to both of these questions could be provided by signaling via a mobile growth factor, as has been proposed on the basis of elegant studies in animals (Day and Lawrence, 2000; Hufnagel et al., 2007). If such a factor is produced from a limited source, for example the very center of the primordium, growth of the organ will change the factor's concentration and/or relative distribution in the primordium. Growth would dilute a highly mobile factor with a largely homogeneous distribution throughout the organ, whereas, for a factor with a more restricted mobility, growth would push cells out of its reach. In either case, this change in relative distribution could then be used to measure the size of the primordium; growth would stop, either if the reduced concentration no longer sustained growth, or as a consequence of the mechanical stress that results when cells outside of the factor's reach stop growing. As the concentration of the factor itself or the mechanical stress would be sensed by all primordium cells in a similar manner, their decision to stop growing could thus be coordinated.

In plants, phytohormones of various classes represent such mobile factors that influence organ growth. For example, cytokinins mainly stimulate cell proliferation, while auxins and brassinosteroids promote both proliferation and expansion (Haubrick and Assmann, 2006; Sakakibara, 2006; Teale et al., 2006). The importance of phytohormones in regulating organ growth is underlined by the growth defects in plants unable to synthesize or perceive a given hormone. Plants insensitive to brassinosteroids, gibberellins, auxins, and cytokinins all show characteristic dwarfing phenotypes, whereas ethylene insensitivity results in larger organs (Dharmasiri et al., 2005; Guzman and Ecker, 1990; Li and Chory, 1997; Peng and Harberd, 1993; Riefler et al., 2006). Besides intercellular signaling by the classical phytohormones, other growth-stimulating signals have also been implicated in organ-size control. The putative transmembrane receptors of the ERECTA (ER) family promote cell proliferation in a partially redundant manner (Shpak et al., 2004) and might thus act as plant growth factor receptors (Ingram and Waites, 2006).

To gain further insight into how the size of plant organs could be measured, we sought to identify genes whose loss and gain of function produce opposite effects on plant organ size. Here, we analyze the role of the *KLUH* (*KLU*) gene from *Arabidopsis* in stimulating plant organ growth. It encodes the cytochrome P450 CYP78A5, whose expression pattern has been described previously, yet no biological function could be assigned in the absence of a loss-of-function mutant (Zondlo and Irish, 1999). Our results suggest that KLU contributes to the generation of a growth-stimulating signal distinct from the classical phytohormones that prevents proliferation arrest, until the correct primordium size has been reached. We suggest a simple geometric model for how *KLU*-dependent signaling could be used to measure the size of growing plant organs.

RESULTS

klu Mutants Show Reduced Growth of Aerial Organs

To identify genes controlling organ size in plants, we screened an EMS-mutagenized population of *Arabidopsis* for alterations in floral organ size. We isolated three independent recessive mutations producing a very similar phenotype of reduced leaf and flower size (Figures 1A and 1B; Figure S1, see the Supplemental Data available with this article online). Complementation tests demonstrated these to be alleles of one locus, which, because of its slight stature, we termed *KLUH* (*KLU*), the inverse of the comic monster Hulk. Homozygous *klu* mutant plants form smaller leaves, sepals, and petals than wild-type, with a comparable reduction of both their length and width, whereas heterozygous *klu-1*/+ plants are indistinguishable from wild-type (Figures 1F–1H). The reduced size of *klu* mutant petals and leaves is not caused by smaller cells, indicating that it is the number of petal and leaf cells that is lower. As with lateral organs, stem thickness is reduced in *klu* mutants (Figure 1I), whereas the rate of root growth is unaffected (data not shown). In addition, *klu* mutants show a slightly reduced apical dominance (Figure S1) and flower 2 days earlier than wild-type plants (Figure 1J). Thus, the *KLU* gene is required for shoot organs to attain their wild-type sizes, promoting growth by cell proliferation.

KLU Encodes a Cytochrome P450 Monooxygenase

We isolated the *KLU* gene by positional cloning. *KLU* corresponds to the annotated transcription unit *At1g13710* (Figure 2). All three mutant alleles from the EMS screen contain typical G-to-A transitions in the *At1g13710* coding sequence, causing premature stop codons. Plants homozygous for a transposon insertion in the first exon of *At1g13710* (SM_3_39145, *klu-4*) show the *klu* mutant phenotype (Figures 1D–1J), and transformation of homozygous *klu* mutants with a genomic copy of *At1g13710* restores a wild-type phenotype (Figure S2I).

At1g13710 encodes the putative microsomal cytochrome P450 monooxygenase CYP78A5, one of six members of the CYP78A family in *Arabidopsis*. As genes are traditionally named on the basis of their loss-of-function phenotypes, we will continue to use *KLU* throughout. As described previously, the KLU protein contains the hallmark features of functional cytochrome P450 enzymes, including a membrane anchor and the conserved heme-binding region with the invariant cysteine for coordinating the iron ion used in catalysis (Zondlo and Irish, 1999). KLU belongs to the A class of cytochrome P450 enzymes, members of which are only found in plants and are thought to perform plant-specific functions (Schuler and Werck-Reichhart, 2003).

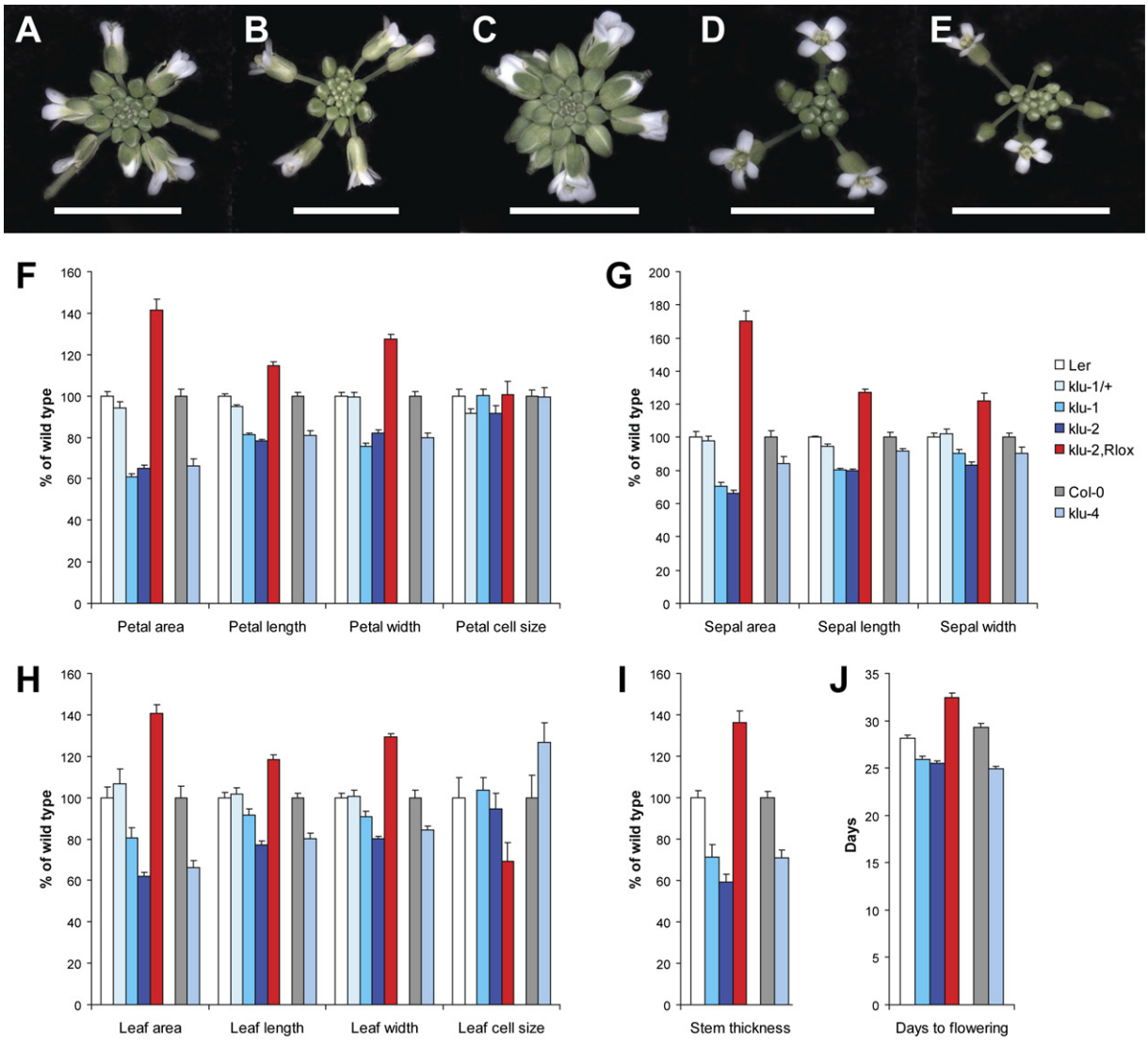

Figure 1. Organ Growth Is Controlled by *KLU* Activity

(A–E) Inflorescences of (A) L*er* control, (B) *klu-2*, (C) the *KLU*-overexpressing line *klu-2*,RLox, (D) Col-0 wild-type, and (E) *klu-4*.

(F–J) Measurements of organ sizes and flowering time for the indicated genotypes: (F) petals, (G) sepals, (H) leaves, (I) stems, (J) flowering time. Values represent mean + SEM (n = 20).

Scale bars are 8 mm.

Overexpression of *KLU* from Its Endogenous Promoter Increases Organ Size

Based on the rescue experiment (see above), we isolated lines that expressed *KLU* at levels comparable to those of wild-type, as well as ones that overexpressed *KLU* (Figures S2H and S2I). Whereas the former are indistinguishable from wild-type, moderately increasing *KLU* activity in its endogenous expression domain (Figures S2F and S2G) causes overgrowth of leaves, sepals, and petals due to increased numbers of cells (Figures 1C and 1F–1H). Stem thickness is also increased in the overexpressing plants (Figure 1I). Thus, *KLU* activity promotes organ growth by cell proliferation, with final organ size depending on the level of *KLU* activity.

KLU Controls the Timing of Growth Arrest

To determine how changes in *KLU* activity lead to altered organ size, we analyzed the growth dynamics of petal primordia in *klu* mutants and the endogenous overexpressors. Compared to wild-type, *klu* mutant petals grow at the same rate, yet they stop growing earlier, leading to a smaller final size (Figure 3A). By contrast, petal primordia in the overexpressing plants continue to grow for a longer time at the same rate as wild-type, causing a larger final size (Figure 3B). To analyze the cellular basis for these growth patterns, we compared cell division rates over time in the epidermis of wild-type and *klu* mutant petal primordia, by using the mitotic marker gene *pAtCycB1;1::CDBGUS* (Donnelly et al., 1999). While cell division rates in

Figure 2. Structure of the *KLU* Gene and Mutant Alleles

Schematic representation of the *KLU* gene; exons are shown as filled rectangles, the intron is shown as an open rectangle, 5′ and 3′ UTRs are shown as crosshatched rectangles, and nontranscribed flanking regions are shown as lines. The molecular lesions in the four loss-of-function alleles are indicated, as is the invariant cysteine that was mutated to inactivate the protein for control lines. The position of the inserted T-DNA in the activation tagging line AT198 is shown relative to the putative TATA box.

young primordia were very similar between *klu* mutants and wild-type, cells in mutant primordia stopped proliferating earlier than those in wild-type, due to both a more rapid decrease in the relative size of the domain in which cells divide (Figure S3A) and to a reduced rate of proliferation within this domain (Figure 3C). Consistent with a premature arrest of proliferation, cells in the blade region of mutant petal primordia entered the phase of postmitotic expansion earlier than those in wild-type (Figure 3D).

To determine when *KLU* acts to stimulate growth, we generated *klu* mutant plants in which *KLU* expression could be induced in developing petal primordia by using the ethanol switch (Deveaux et al., 2003). After a short (less than 24 hr) pulse of *KLU* mRNA expression (Figure S3C), petals were allowed to mature and their size was determined. Whereas inducing *KLU* expression in late stages of flower development (2 days before bud opening; right side of Figure 3E) causes a weak, but reproducible defect in petal expansion, *KLU* induction is most efficient in promoting petal growth at around 6 days before flower opening (Figure 3E). This enlargement is due to an increased cell number, because cell size is not affected (290 ± 5 μm^2 for induced petals, 301 ± 7 μm^2 for uninduced petals). Six days before bud opening corresponds to the time when cell division rates in petal primordia fall rapidly and the difference between *klu* mutant and wild-type petals in cell proliferation starts to become apparent (highlighted in gray in Figure 3C). YFP fluorescence from a linked reporter that monitors the ethanol induction is detected at high levels in petals of all growth stages (inset in Figure 3E), suggesting that the effect is not due to preferential transgene induction in the responding petals, but indeed reflects different sensitivities of petal primordia to *KLU* action. Together with the difference in cell proliferation in *klu* mutant and wild-type petals, this suggests that *KLU* acts late during the proliferation phase.

Thus, *KLU* activity regulates the timing of organ growth, preventing a premature arrest of growth by proliferation.

The *KLU* Expression Domain Is Distinct from Regions of Organ Proliferation

Based on mRNA in situ hybridization (Figure S2; Zondlo and Irish, 1999) and a *pKLU::GUS* reporter (Figures 4A, 4C, and 4D), *KLU* is expressed on the flanks of the shoot meristem, at the base of developing leaves, and later on at the boundary of the shoot and lateral organs. The same basic pattern is observed in developing flowers (Figures 4D and 4E; Figures S2C–S2E). Specifically, in petals, expression is initiated uniformly in very young primordia (Figure S2C), before it becomes restricted to the periphery of the organ (Figures 4E–E″) and eventually ceases (data not shown). A similar expression at the tip of the organ is seen in stamen primordia; expression is also found at the base of integuments (Zondlo and Irish, 1999).

Leaves in dicotyledonous plants grow by dispersed cell divisions throughout the prospective blade region, before two consecutive cell cycle arrest fronts terminate proliferation in a basipetal orientation (Donnelly et al., 1999; Nath et al., 2003; White, 2006). Also in petals, cell proliferation occurs throughout the distal region that will form the blade (Figure 4F). When comparing the regions of *KLU* expression and cell division in young leaves (Figures 4A and 4B) and in petals at the stage that is most sensitive to *KLU* activity (Figures 4E′ and 4F′; cf. above), it is apparent that *KLU* is expressed outside the region of proliferation. Of note, *KLU* expression is still detected when cells have already ceased to proliferate (Figures 4E″ and 4F″), suggesting that it is not the downregulation of *KLU* expression that causes the eventual arrest of proliferation. Together, these results suggest that *KLU* acts non-cell-autonomously to promote organ growth.

KLU Protein Localizes to the ER and Does Not Move into Regions of Growth

Given its possible non-cell-autonomous role, we asked whether KLU protein itself may be a mobile signal. To localize KLU protein, we expressed a KLU-vYFP fusion protein under the control of the *KLU* promoter. The fusion protein fully complemented the *klu* mutant phenotype, demonstrating its functionality (Figure S2I). Intracellularly, vYFP fluorescence was detected in a ring around the nucleus and in a mesh-like pattern in the cytoplasm (Figure 4I), which is typical for ER-associated proteins (Haseloff et al., 1997). Association with the ER is expected to strongly reduce a protein's cell-to-cell mobility, and, indeed, KLU-vYFP fusion protein was only detected at the base and around the perimeter of the apical domain in developing petals, not in the petal center (Figures 4G and 4H). Thus, KLU protein does not appear to move from the cells that express it into the regions of cell proliferation, suggesting that the mobile growth-promoting signal is downstream of KLU activity.

Petal-Specific Activation Tagging of *KLU* Increases Not Only the Size of Petals, but Also the Size of Sepals

In a complementary approach to the loss-of-function screen for organ-size regulators, we performed petal- and

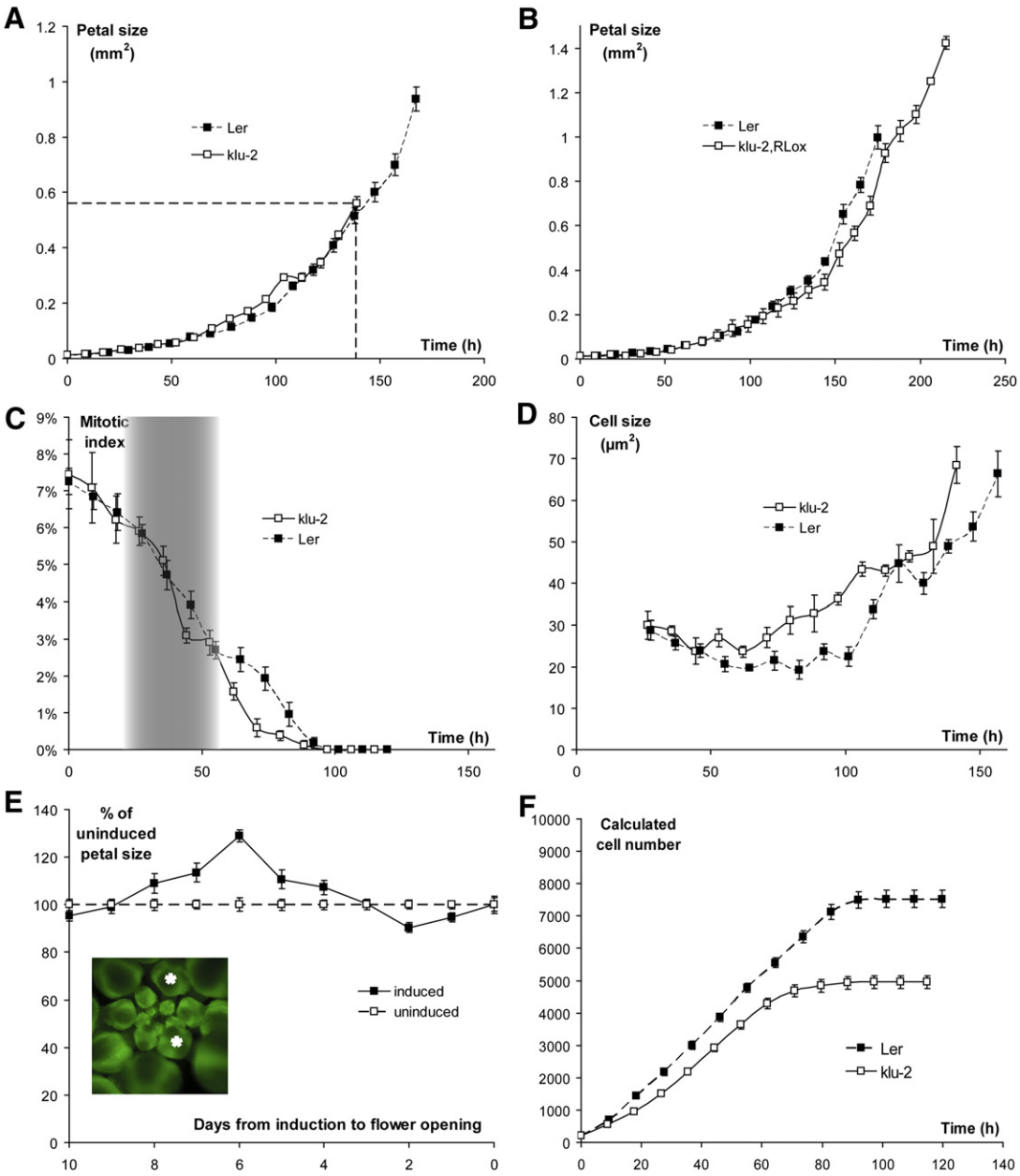

Figure 3. *KLU* Regulates the Timing of Proliferative Growth

(A–E) For clarity, the curves in (A), (B), and (D) end at the oldest unopened flowers, which mirror final organ sizes, because final cell expansion at flower opening is indistinguishable in all genotypes. (A and B) Developmental series of petal growth in (A) *klu-2* and (B) the overexpressing line *klu-2*,RLox compared to wild-type. (C and D) Developmental series of (C) cell division activity and (D) cell size in *klu-2* mutant versus wild-type petal primordia. The gray region in (C) marks the time window of maximum sensitivity to *KLU* activity, as determined by the pulse experiment in (E). The mitotic index in (C) is calculated relative to the cell number in the area in which cells proliferate. (E) *KLU* acts at the end of the proliferation phase. The diagram shows the final size of petals that received a pulse of *KLU* activity (by EtOH induction of *klu-2*, *pAP3::AlcR-AlcA::KLU-AlcA::vYFPer* plants) at different times during their development (measured by days until flower opening). Values are relative to the sizes of uninduced petals collected at the same times. The inset shows YFP fluorescence from the linked *AlcA::vYFPer* reporter at 24 hr after induction. The asterisks mark flowers ~6 days before opening. (F) Calculated numbers of epidermal cells in petals of L*er* and *klu-2* plants (see the Supplemental Data for details).
Values represent mean + SEM (n = 10).

stamen-specific activation tagging. A line expressing the synthetic transcription factor LhG4 (Moore et al., 1998) in petals and stamens was transformed with a T-DNA harboring six copies of the cognate *pOp* enhancer next to its right border (see Experimental Procedures), and primary transformants were screened for changes in petal size.

Line AT198 shows reduced petal expansion in early-developing flowers and larger than wild-type petals in later

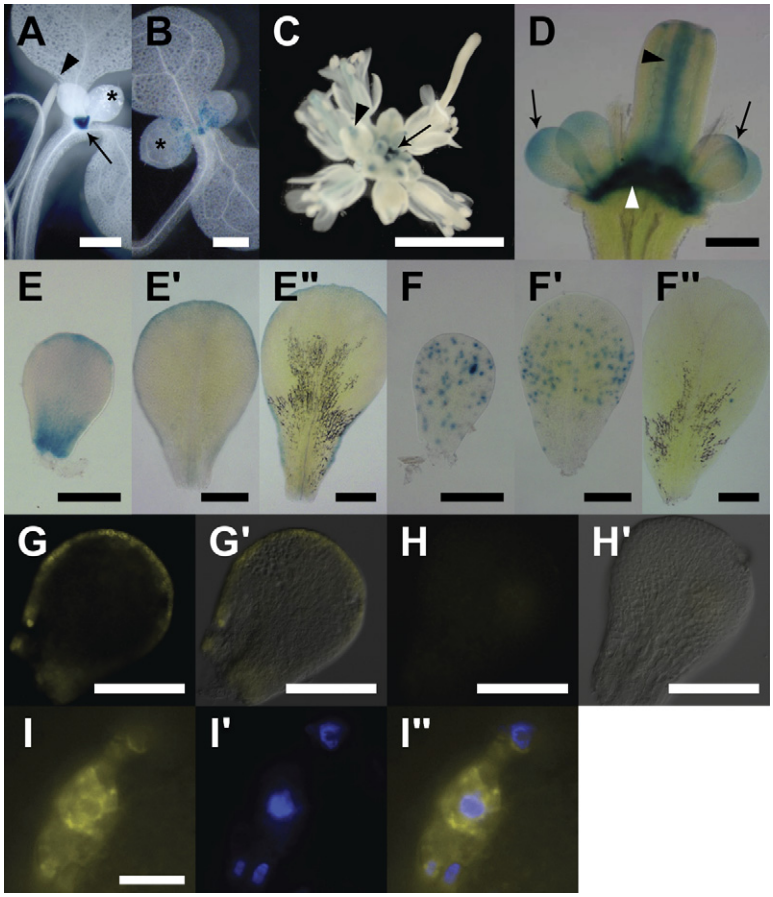

Figure 4. *KLU* Expression Pattern and Protein Localization

(A–F″) (A and C–E) *pKLU::GUS* expression pattern. GUS activity is visualized by blue staining. (A) In seedlings, *KLU* is expressed in the shoot apex region and at the base of growing leaves (arrow), but not in growing leaves or in the root meristem (arrowhead). (B) A *pAtCycB1;1::CDBGUS*-expressing seedling shows GUS staining at the apex and in the basal region of developing leaves. The asterisks in (A) and (B) indicate leaves of comparable age. (C) In the inflorescence, *KLU* expression is detected in young flower primordia (arrow) and in the gynoecia of older flowers (arrowhead). (D) A developing flower with sepals and stamens removed. *KLU* is expressed in the base of the flower (white arrowhead), in the gynoecium (black arrowhead), and in growing petals (arrows). (E–E″) *KLU* expression is seen at the base and around the periphery of growing petals (E′ and E″). (F–F″) Cell division activity, as monitored by the *pAtCycB1;1::CDBGUS* reporter, occurs initially (F) throughout the petal, before it (F′) ceases at the base and tip of the organ and (F″) ultimately arrests entirely. Petals in (E) and (F), in (E′) and (F′), and in (E″) and (F″) are from flowers in comparable developmental stages.

(G–I″) KLU protein localization, as determined by YFP fluorescence microscopy on complemented *pKLU::KLU-vYFP, klu-2* plants. (G) The KLU-vYFP fusion protein is only detected around the periphery of developing petals, whereas the central region only shows the background fluorescence of petals from nontransgenic plants (compare with [H]). (H) Background fluorescence from petals of nontransgenic plants. (G′) and (H′) show merged images of the YFP fluorescence shown in (G) and (H), respectively, with bright-field images of the same petals. (I) Intracellularly, KLU protein is associated with the ER. KLU-vYFP fluorescence is detected in a ring around the nucleus (DAPI staining in [I′] and merged image in [I″]) and in a mesh-like pattern throughout the cytoplasm.

Scale bars are 500 μm in (A) and (B), 1 cm in (C), 100 μm in (D)–(F), 50 μm in (G) and (H), and 5 μm in (I).

flowers, with an increase in both length and width (Figures 5A–5C and 5G). The reduced petal size in early flowers is accounted for by a failure of petal cells to expand, while the enlarged petals of later-developing flowers are due to more and somewhat larger cells (Figure 5G). Surprisingly, not only petals, but also sepals are enlarged in AT198, both in early- and late-developing flowers (Figure 5H), again due to a combination of more and larger cells.

The tagging T-DNA in line AT198 is inserted into the 5′ upstream region of *KLU*, with the six copies of *pOp* located 115 bp 5′ to the presumed TATA box (Figure 2), suggesting that ectopic expression of *KLU* is responsible for the increased organ size.

We confirmed this by transforming the starting line *pAP3::LhG4* with a direct *pOp::KLU* construct. Doubly transgenic plants recapitulate the phenotype of AT198 and form smaller petals in early-developing flowers and larger than wild-type petals in later flowers, as well as larger sepals (Figures 5D, 5E, 5G, and 5H). Given that the pulse experiment (cf. above) suggested that *KLU* activity in late stages of petal growth interferes with cell expansion, we believe the opposite effects on petal size to be due to slight differences in the timing of *pAP3* expression, with *pAP3* staying active for longer in flowers that arise early.

To test whether the presumed activity of KLU as a cytochrome P450 is required for its function in planta, we mutated the invariant Cys residue that coordinates the iron ion in the heme of cytochrome P450s (Groves, 2004) and expressed this mutated protein under the control of the *pAP3* promoter (Figure 5F). This did not alter floral organ sizes (Figures 5G and 5H), suggesting that enzymatic activity of KLU is required to stimulate growth.

Thus, ectopic expression of *KLU* in petals and stamens increases not only the size of petals, but also that of neighboring sepals.

Ectopic *KLU* Expression Stimulates Sepal Overgrowth in a Non-Cell-Autonomous Manner

To confirm that the *pAP3* promoter that we used was specifically expressed in developing petals and stamens, we performed mRNA in situ hybridization, with either a *KLU* antisense probe on AT198 tissue or a *vYFP* antisense probe on tissue of a *pAP3::LhG4; pOp::KLU-vYFP* recapitulation line (Figures 5I and 5J). Strong ectopic expression

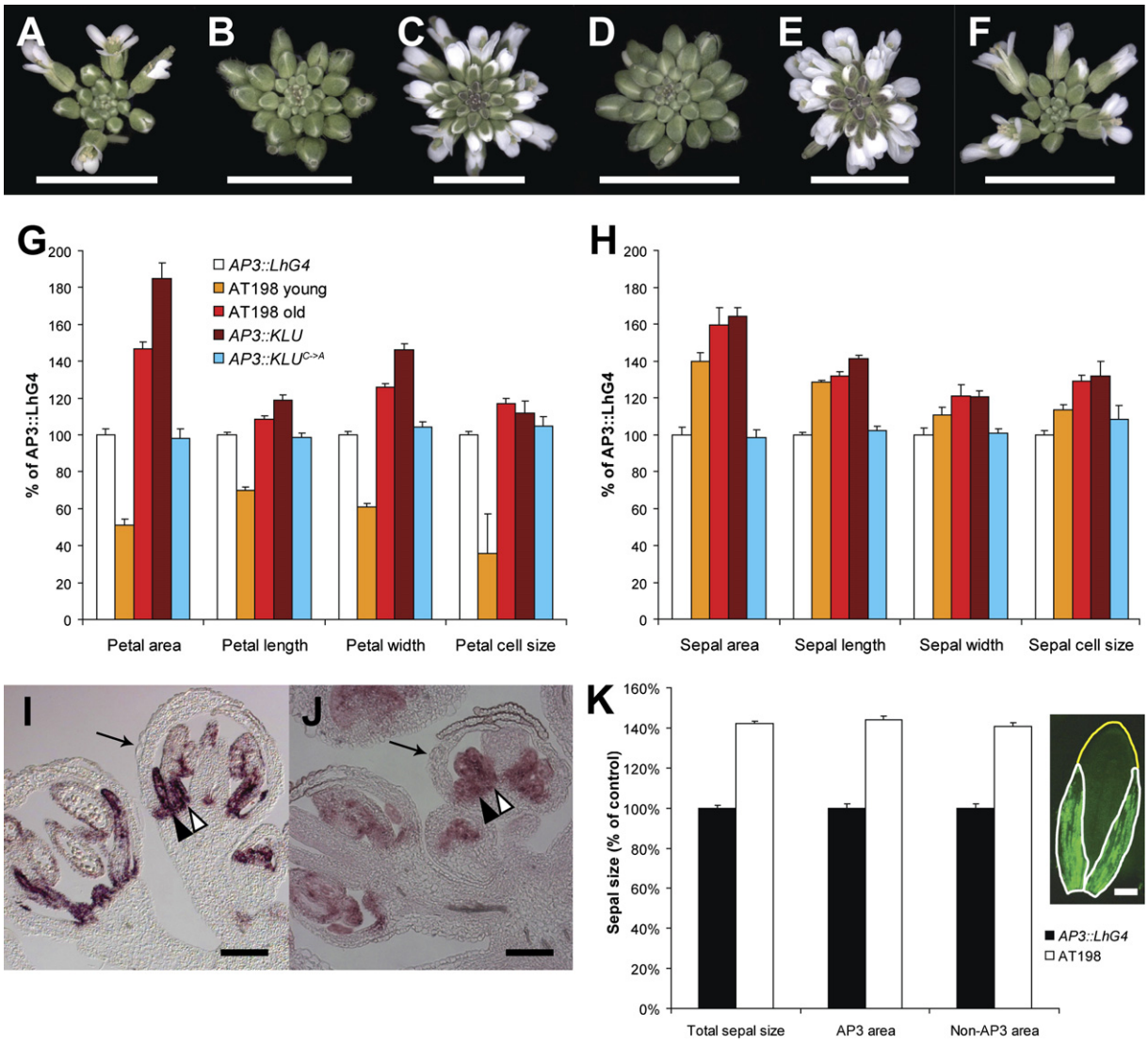

Figure 5. Activation Tagging of *KLU* in Line AT198

(A–F) Inflorescences. (A) *pAP3::LhG4* control line. (B and C) An AT198 plant (B) shortly after bolting and (C) later in development. (D and E) A recapitulated *pAP3::KLU* plant (D) shortly after bolting and (E) later in development. (F) A plant expressing the mutated *KLU* protein from the *pAP3* promoter (*pAP3::KLU*$^{C\rightarrow A}$).

(G and H) Measurements of (G) petals and (H) sepals of the genotypes shown in (A)–(F).

(I and J) In situ hybridizations with (I) *KLU* and (J) *vYFP* antisense probes to inflorescence sections of (I) line AT198 and an (J) *AP3::KLU-vYFP*-expressing plant show expression in developing petals (black arrowheads) and stamens (white arrowheads), but not in sepals (arrows), except for a small region at their base.

(K) Measurements of total sepal size and the areas occupied by descendants of cells that did ("AP3 area") or did not ("Non-AP3 area") express the *pAP3* promoter during early stages of sepal development from control and AT198 plants. The inset shows a representative sepal with descendants of *pAP3*-expressing cells marked by YFP fluorescence. The areas bounded by the white lines represent the "AP3 area," whereas the remainder of the sepal (yellow outline) is the "Non-AP3 area."

Values represent mean + SEM (n = 20). Scale bars are 8 mm in (A)–(E), 100 μm in (I) and (J), and 200 μm in (K).

was detected in petal and stamen primordia, but not in developing sepals, except for in a small region at the very base of sepal primordia, as described for the endogenous *AP3* gene (Tilly et al., 1998). Thus, sepal overgrowth occurs without widespread *KLU* expression in sepal primordia.

However, it is still possible that the increase in sepal size is not due to a genuinely non-cell-autonomous effect; it might merely be caused by excess growth of the descendants from basal sepal cells that expressed the *pAP3::KLU* transgene at one point. To distinguish between such a "memory" effect or a truly non-cell-autonomous action of *KLU*, we analyzed the growth of sepal cells that had expressed the *pAP3* promoter. In control and AT198 plants, *pAP3*-expressing cells and their mitotic daughters

were marked by CRE/*loxP*-mediated recombination, yielding a cell-autonomous *35S::vYFPer* reporter. Sepals were analyzed when the areas containing YFP-positive clones reached the maximum size in both genotypes (Figure 5K; Figure S4). Overall, sepals from AT198 were 42% larger than control sepals. This was due to a 40% larger region of unmarked cells and a 44% increase in the area occupied by descendants of formerly *pAP3*-expressing cells. Thus, sepal cells that have at one point activated the *pAP3* promoter and ones that have not show similar overgrowth in AT198 sepals, demonstrating that *KLU* activity indeed promotes growth at a distance.

The Transcriptional Response to *KLU* Activity Is Distinct from Phytohormone Responses

Together, the above-described results suggest that KLU is involved in generating a mobile, growth-promoting signal molecule. Obvious candidates for this are the classical phytohormones. To test whether *KLU* might be involved in modulating the level of one of these, we defined *KLU*-responsive genes by transcriptional profiling and compared these to the described sets of hormone-regulated genes (Nemhauser et al., 2006), as a proxy for determining hormone levels directly. We generated *klu-2* mutants carrying a construct for inducible overexpression of wild-type KLU protein or of the inactive Cys-to-Ala mutant as a negative control, and we compared the transcriptional profiles of inflorescences at 1.5 and 4 hr after induction by using two independent transgenic lines per construct. *KLU*-responsive genes were defined as those that were significantly up- or downregulated after induced overexpression of the wild-type protein (both compared to untreated plants and to induced overexpressors of the mutated protein), but were not significantly changed after induced overexpression of the mutated protein. These stringent criteria identified 78 genes as up- and 9 genes as downregulated by *KLU* activity (Tables S1 and S2). As the described lists of hormone-responsive genes are based on treated seedlings (Nemhauser et al., 2006), we assessed the overlap between *KLU*-responsive genes in inflorescences and seedlings by RT-PCR. Of 12 randomly chosen genes that were regulated by *KLU* activity in inflorescences, 11 showed the same response in seedlings (Figure S5), indicating that the response to *KLU* activity is not strongly influenced by the developmental stage of the shoot and allowing for a meaningful comparison of our list of *KLU*-responsive genes to the published sets of hormone-responsive genes. These RT-PCRs also suggested that the mutated protein is not entirely inactive, because strongly responding genes also showed weak induction in plants overexpressing the mutated protein.

When comparing the *KLU*-responsive gene lists to the published sets of hormone-responsive genes (Nemhauser et al., 2006), no clear overlap between *KLU* up- and downregulated genes and the gene sets controlled by any of the classical phytohormones is seen (Table 1). Also, we used RT-PCR to analyze hormone-induced markers (two genes per hormone) after induction of *KLU* activity in seedlings (Figure S6): if changes were observed, these were either not specific to expression of the active KLU protein (e.g., *At2g42540*), or they were not consistent between the two genes tested for one hormone (e.g., *At2g40610* and *At5g57560*). Thus, the lack of consistent overlap between the transcriptional responses to KLU and to phytohormones strongly suggests that KLU does not directly contribute to biosynthesis or degradation of one of the classical hormones.

As responses to hormones do not necessarily involve transcriptional regulation, we used a complementary approach to address the relationship of *KLU* and phytohormones. To test whether the mutant phenotype could be rescued or exacerbated by treatment with exogenous hormones, leaf size was compared after growing wild-type and *klu-2* mutant seedlings on media with different hormone concentrations (Figure S7). Compared to growth on control medium, the relative difference between wild-type and *klu-2* mutant leaves was not significantly altered by any of the treatments. This lack of interaction between the *klu* mutation and exogenous phytohormones provides further support for the notion that *KLU* does not modulate the levels of the hormones tested.

Double Mutant Analysis

To determine whether *KLU* is involved in a previously defined pathway regulating organ size, we analyzed the phenotypes of double mutants. BB limits the phase of proliferative growth, whereas ANT prolongs it (see above). *ANT* activity is stimulated by the auxin-inducible *ARGOS* gene. Similarly, the presumed transcriptional coactivator AN3 promotes organ growth by proliferation.

Eliminating *KLU* function in a *bb*, *ant*, or *an3* mutant or in a *35S::ARGOS*-overexpressing background in all cases caused the same relative decrease in organ size as in the corresponding control (Figures 6A–6D). Thus, the requirement for *KLU* activity is not modified by any of the other genes tested, arguing for independent genetic pathways.

The cytochrome P450 *ROT3* stimulates growth in the longitudinal direction by contributing to the synthesis of brassinosteroids (Kim et al., 2005). Loss of *KLU* function in a *rot3* mutant background caused the same relative size reduction as in control plants, suggesting independent activities (Figure 6E). Given that *ARGOS*/*ANT* and *ROT3* act in auxin- and brassinosteroid-mediated growth control, respectively, this lack of genetic interaction further supports the independence of *KLU* from auxins and brassinosteroids.

Lastly, the *klu-4* mutant allele in the Col-0 background with a functional *ER* gene shows the same reduction in organ sizes as the *klu-1* and *klu-2* alleles in the L*er* background, which is mutant for *er* (Figures 1F–1J), arguing against an interaction between *KLU* and the *ER* signaling pathway.

DISCUSSION

KLU Stimulates Organ Growth by Preventing a Premature Arrest of Proliferation

We have identified the *Arabidopsis* cytochrome P450 *KLU* as a regulator of plant organ growth. While loss of *KLU*

Table 1. Comparison of *KLU*-Regulated and Phytohormone-Responsive Genes

	Total	Overlap	KLU Upregulated (Total)	Overlap	KLU Downregulated (Total)
ABA upregulated	1440	6 (5)	78	2 (1)	9
ACC upregulated	167	1 (1)	78	0 (0)	9
BL upregulated	268	1 (1)	78	1 (0)	9
CK upregulated	332	1 (1)	78	0 (0)	9
IAA upregulated	430	6 (1)[a]	78	0 (0)	9
MJ upregulated	806	5 (3)	78	0 (0)	9
GA upregulated	40	0 (0)	78	1 (0)[a]	9
ABA downregulated	1476	1 (5)	78	1 (1)	9
ACC downregulated	365	1 (1)	78	2 (0)[a]	9
BL downregulated	383	0 (1)	78	0 (0)	9
CK downregulated	163	1 (1)	78	1 (0)[a]	9
IAA downregulated	355	1 (1)	78	2 (0)[a]	9
MJ downregulated	701	3 (2)	78	0 (0)	9
GA downregulated	82	0 (0)	78	1 (0)[a]	9

The sets of *KLU* up- or downregulated genes were compared to those of the hormone-responsive genes (Nemhauser et al., 2006) by using a chi-square test. Values in the third and sixth columns indicate the observed overlap, and values in parentheses indicate the overlap expected by chance. Bonferroni correction was applied separately to all comparisons with *KLU* up- and *KLU* downregulated gene sets. ABA, abscisic acid; ACC, 1-aminocyclopropane-1-carboxylic acid (an ethylene precursor); BL, brassinolide; CK, cytokinin; IAA, indole-3-acetic acid; MJ, methyl-jasmonate; GA, gibberellic acid.
[a] p values less than 0.01.

function leads to smaller lateral organs, its overexpression is sufficient to cause organ overgrowth. In both cases, the effect is largely due to altered numbers of cells, rather than changes in cell size.

In order to gain a more detailed understanding of *KLU* function in organ growth, we calculated epidermal cell numbers in wild-type and *klu* mutant petals using the measured cell proliferation activity (cf. Supplemental Data). These calculations show that cell proliferation ceases earlier in *klu* mutant petals than in wild-type petals, both in terms of time (Figure 3F) and the accumulated total cell number (Figure S8A). Cell numbers in *klu* mutant organs follow a logistic growth law (Figures S8B and S8C; Supplemental Data). This suggests a cell-number-dependent feedback mechanism, for example the accumulation of an inhibitor of cell proliferation. *KLU* activity in wild-type appears to counteract this process, leading to a less rapid decline of proliferation at later stages (Figures S8B and S8C). Due to the precocious arrest of proliferation, mutant petals reach only about 66% of the wild-type cell number. Given that *klu* mutant and wild-type cells expand to approximately the same size (Figure 1F), this effect accounts for the observed difference in final organ size.

A role of *KLU* mainly toward the end of the proliferation phase is supported by our pulse experiment. At this stage, petal primordia are most sensitive to a short pulse of *KLU* activity, which causes a substantial proportion of cells to divide once more. By contrast, primordia that experienced the induction pulse earlier during their development (left side of Figure 3E) reacted less strongly or not at all, suggesting that the requirement for *KLU* increases toward the end of the proliferation phase. Consistent with the very similar growth behavior of *klu* mutant and wild-type petals at the beginning of our measurements (Figure 3; Figure S8), this may be due to the action of additional growth stimulators that are dominant over and thus effectively mask *KLU* function during the initial phase of proliferation, but whose activity declines early, revealing the requirement for *KLU* afterward.

Thus, our calculations and experiments both support the hypothesis that *KLU* functions mainly toward the end of the proliferation phase to prevent its premature arrest.

A Distinct Pathway for Controlling Organ Size

The function of *KLU* in maintaining growth by proliferation is similar to the roles of *ANT*, *ARGOS*, and *AN3*, and antagonistic to *BB*. However, the respective double mutants indicate that *KLU* acts independently of these four genes. The very similar effects of the *klu* mutant alleles in backgrounds with and without a functional *ER* gene also argue for genetic independence, as do the clearly distinct phenotypes of *klu* and *jag* or *jag nub* double mutants (Dinneny et al., 2004, 2006; Ohno et al., 2004). Thus, *KLU* appears to define a size-control pathway that is distinct from ones described previously.

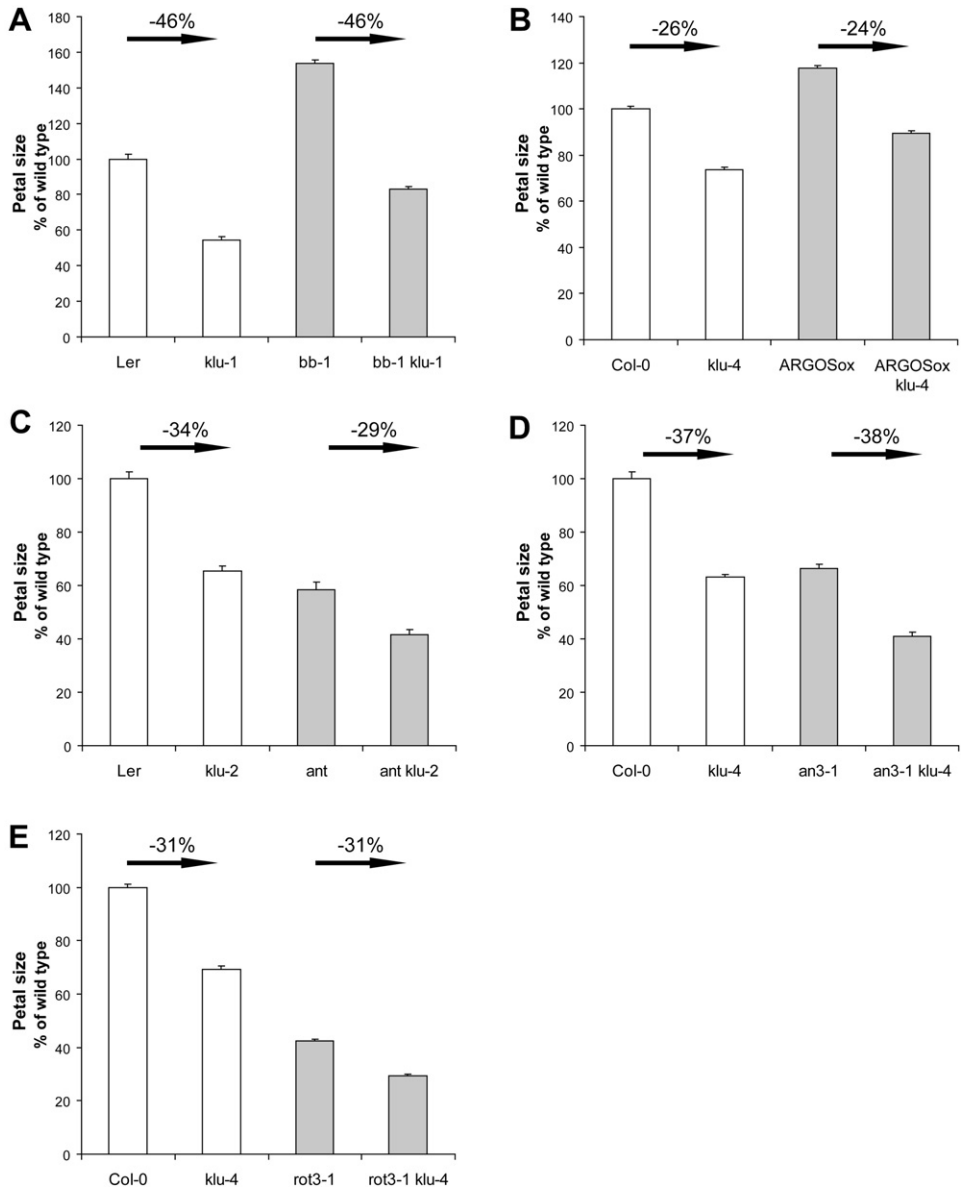

Figure 6. Double Mutant Analysis

(A–E) Double mutants of (A) *klu-1 bb-1*, (B) *klu-4 35S::ARGOS* (*ARGOSox*), (C) *klu-2 ant*^{72F5}, (D) *klu-4 an3-1*, and (E) *klu-4 rot3-1* all show additive phenotypes as assessed by petal size.

Values represent mean + SEM (n = 20).

KLU Is Involved in Generating a Novel Mobile Growth Signal

Our findings suggest that KLU is involved in generating a growth-stimulating compound that is mobile and distinct from the classical phytohormones. This notion is based on the following observations: first, the discrepancy between the *KLU* expression domain and the region of cell proliferation in organ primordia suggests that *KLU* acts non-cell-autonomously. Second, *KLU* overexpression in petals and stamens increases not only the size of petals, but also that of the adjacent sepals, and this sepal enlargement is at least partly due to overgrowth of cells that have never detectably activated the transgene. Third, a functional KLU-vYFP fusion protein does not show detectable protein movement from the expressing cells in the organ periphery into the region of cell proliferation, suggesting that the mobile growth-stimulating activity is downstream of KLU protein function. Fourth, growth stimulation by KLU is disrupted by mutating an invariant cysteine that serves to coordinate the catalytic iron ion in the heme group of functional cytochrome P450s, suggesting that growth promotion requires enzymatic activity of KLU. Fifth, the

sets of *KLU*-responsive genes show essentially no overlap with the defined sets of phytohormone-responsive genes, and the *klu* mutant phenotype is neither rescued nor exacerbated by treating with exogenous hormones, strongly suggesting that KLU does not modulate the levels of known phytohormones.

It was proposed previously that members of the CYP78A family of cytochrome P450s are involved in the generation of novel signaling compounds. Overexpression of CYP78A9 in developing flowers caused altered gynoecium growth and parthenocarpy (Ito and Meyerowitz, 2000). Mutations in the rice *PLASTOCHRON1* (*PLA1*) gene, encoding CYP78A11, reduce the size of leaves and the length of the plastochron, i.e., the time between the production of successive primordia by the shoot meristem (Miyoshi et al., 2004). Because of the restricted expression domain of *PLA1* at the base of developing leaves, a role for *PLA1* in the synthesis of a mobile signal was suggested. As *klu* mutants also show a weak, but reproducible acceleration of the plastochron (data not shown), the phenotypes of *klu* and *pla1* mutants appear very similar, suggesting that they function in the biosynthesis of a conserved plant signaling molecule.

Which molecule(s) could this be? Cytochrome P450s are heme-dependent monooxygenases that catalyze a wide variety of reactions on organic compounds, such as hydroxylations, epoxidations, etc. (Schuler and Werck-Reichhart, 2003). They are involved both in detoxification of xenobiotics and in the biosynthesis of structural components and signaling molecules, e.g., phytohormones or the branching inhibitor produced by the *MAX* pathway (Booker et al., 2005). The closest homolog to KLU with a described catalytic activity is the maize CYP78A1 protein, which, in vitro, catalyzes omega-hydroxylation of a fatty acid (Imaishi et al., 2000). Also, eight of the nine cytochrome P450 genes that are transcriptionally regulated by *KLU* activity (CYP76C1, 76C2, 76C3, 76C4, 81F3, 86A4, 86A7, and 94B1) are linked to fatty-acid modification: omega-hyroxylation of fatty acids has been directly shown for CYP86A4 and CYP86A7 (Duan and Schuler, 2005), whereas CYPs 76C1, 76C2, 76C3, 76C4, 81F3, and 94B1 are all closely related to proteins for which fatty-acid hydroxylation has been shown (Cabello-Hurtado et al., 1998; Pinot et al., 2000; Tamaki et al., 2005). The homology to CYP78A1 and the concerted regulation of these genes by KLU suggest that KLU itself modifies a fatty-acid-related molecule, which could then feedback regulate other enzymatic activities in a biosynthetic pathway.

We note that the characteristic spatial dynamics of cell proliferation do not seem to be affected by *KLU*, e.g., cell proliferation terminates first at the tip and base of the petal primordium in both wild-type and *klu* mutants, and that there are no obvious gradients in proliferation toward the periphery of wild-type petals (Figure S3B). This suggests that the *KLU*-dependent signal is mobile enough to produce a largely homogeneous distribution throughout the primordium, which modulates the rate and timing, yet not the spatial pattern, of proliferation.

A Common Strategy for Measuring Primordium Size Based on Allometry?

How developing organ primordia measure their size to decide when to stop growing is a fascinating, yet poorly understood problem, particularly so in plants. The expression dynamics and non-cell-autonomous function of *KLU* suggest a model of how *KLU* loses its effect on primordium cells as a result of its growth-promoting activity (cf. Supplemental Data). In both petals and leaves, *KLU* is expressed in a limited domain (around the periphery or only at the base, respectively), which for geometrical reasons increases more slowly than the organ as a whole. It is plausible to assume then that the proposed *KLU*-dependent signal will be diluted as the organ grows. As soon as its concentration falls below a critical value, it will no longer be able to sustain cell proliferation, which will then stop. Thus, in this view, the level of the *KLU*-dependent signal reflects the size of the primordium, and its gradual dilution due to growth ensures that cells cease to proliferate and begin expanding when a certain primordium size has been reached.

In *Drosophila* wings, the mobile growth factor Decapentaplegic (Dpp) is secreted along a line of cells in the center of the wing imaginal disc to stimulate proliferation throughout the primordium. As the organ grows, the ratio of Dpp-secreting to -receiving cells decreases for geometrical reasons. Although the exact mechanism is still a matter of debate, this is widely believed to determine up to which size the wing primordium can grow (Day and Lawrence, 2000; Hall, 2004; Hufnagel et al., 2007). Thus, despite obvious differences in the molecular implementation, plants and animals may ultimately use a common strategy for size control based on allometry: a signal source that grows more slowly than the primordium as a whole can only sustain proliferation up to a certain point, ensuring that growth terminates once the organ has reached its target size.

EXPERIMENTAL PROCEDURES

Plant Material and Growth Conditions

The *klu-1*, *klu-2*, and *klu-3* alleles were isolated from an EMS-mutagenized population in the *bb-1* background and backcrossed three times to L*er* plants to isolate *klu* single mutants. The *klu-4* allele was identified from the SM collection of transposon insertion lines (Tissier et al., 1999). *bb-1* and *ant*^{72F5} mutants have been described by Disch et al. (2006). *rot3-1* and *an3-1* alleles were obtained from the Nottingham *Arabidopsis* Stock Centre. *35S::ARGOS* plants were provided by Prof. Nam-Hai Chua (Rockefeller University, New York).

Plant growth conditions were as described by Disch et al. (2006). Details of hormone treatments can be found in the Supplemental Data.

Positional Cloning of *KLU*

The *klu-1* mutation was mapped in a *klu-1* × Col-0 F2 population, by using described markers (http://carnegiedpb.stanford.edu/publications/methods/ppsuppl.html) and ones designed from published information (Jander et al., 2002). Sequencing of candidate genes identified point mutations in the *At1g13710* gene in all three *klu* mutant alleles.

Phenotypic Analysis

Organ and cell sizes, as well as growth dynamics of petals, were measured as described by Disch et al. (2006). Values are represented as

mean + SEM (bar charts) or mean ± SEM throughout. Each value corresponds to at least ten petals from at least five plants.

Molecular Cloning

Constructs for plant transformation were generated, and plant transformation was performed by using standard techniques. Detailed information about the constructs used can be found in the Supplemental Data.

Time Window of *KLU* Action in Petal Growth

Homozygous mutant *klu-1* plants containing a *pAP3::AlcR-AlcA::KLU-AlcA::vYFPer* construct were induced by EtOH vapor for 4 hr as described by Deveaux et al. (2003). Petals of stage-14 flowers from five induced and five uninduced plants were measured at the indicated time points after induction.

In Situ Hybridization and GUS Staining

In situ hybridization and GUS staining were performed as described by Disch et al. (2006).

Activation Tagging

Plants homozygous for a kanamycin-resistant *pAP3::LhG4* driver line (a gift from Dr. Yuval Eshed, Weizman Institute, Rehovot, Israel) were transformed with the tagging vector ML450. Backcrossing of transformants to the original *pAP3::LhG4* line was used to confirm that observed phenotypes were dominant. Plasmid rescue of neighboring plant sequences was done as described by Weigel et al. (2000).

Clonal Analysis of *pAP3*-Expressing Sepal Cells

We crossed transgenic plants harboring the *pAP3::CRE-GR* construct with ones carrying the ML988 target construct (cf. Supplemental Data). F1 progeny with both constructs were then crossed to *pAP3::LhG4/pAP3::LhG4; AT198*/+ plants. F1 progeny from this cross were induced by spraying with 5 μM dexamethasone at the time of bolting to strongly activate the CRE recombinase. Clones were monitored in sepals of ten plants with the AT198 phenotype and in ten control plants. Detailed measurements were taken at 11 and 14 days after induction when clone sizes reached their maximum. We defined the area occupied by descendants of cells expressing the *pAP3* promoter in early sepal stages (AP3 area in Figure 5K) as the entire area bounded by a perimeter line connecting the YFP-positive clones in the basal sepal margins and took the non-AP3 area as the remainder of the sepal.

Microarray Analysis

We generated homozygous *klu-2* mutants carrying *35S::AlcR-AlcA::KLU* or *35S::AlcR-AlcA::KLU*$^{C\rightarrow A}$ transgenes. Plants of two independent lines per construct were induced by EtOH vapor for 1.5 and 4 hr, after which RNA was extracted from pooled inflorescences with young flower buds. RNA was also extracted from inflorescences of uninduced *35S::AlcR-AlcA::KLU; klu-2* plants. Hybridization of Affymetrix ATH1 arrays was performed by ServiceXS, Leiden, The Netherlands.

Arrays were quantile normalized by using the RMA method in the R BioConductor version 1.7 packages affy and limma (Gentleman et al., 2004). Differential expression was determined by using the Rank Products method (Breitling et al., 2004).

KLU-responsive genes were defined as genes that were significantly differentially expressed (FDR $\leq$ 0.05) in induced *35S::AlcR-AlcA::KLU; klu-2* plants compared to untreated plants and in induced *35S::AlcR-AlcA::KLU; klu-2* plants compared to induced *35S::AlcR-AlcA::KLU*$^{C\rightarrow A}$*; klu-2* plants, but not in induced *35S::AlcR-AlcA::KLU*$^{C\rightarrow A}$*; klu-2* plants compared to noninduced controls.

Clustering was carried out by using the implementation of Figure of Merit (Yeung et al., 2001) and K-means clustering (Soukas et al., 2000) in TIGR MeV 4.0 (Saeed et al., 2006).

Supplemental Data

Supplemental Data include a mathematical analysis of the role of *KLU* in growth control, Supplemental Experimental Procedures, eight figures, and two tables and are available at http://www.developmentalcell.com/cgi/content/full/13/6/843/DC1/.

ACKNOWLEDGMENTS

We are grateful to Yuval Eshed for providing the *pAP3::LhG4* line, to Nam-Hai Chua for *35S::ARGOS* seeds, and to Gregory van Duyne for the *CRE* plasmid. We thank Enrico Coen, Robert Sablowski, Isabel Bäurle, and members of the M.L. laboratory for helpful comments on the manuscript. This work was supported by a grant from the Deutsche Forschungsgemeinschaft and a David Phillips Fellowship from the Biotechnology and Biological Sciences Research Council to M.L.

Received: May 3, 2007
Revised: August 9, 2007
Accepted: October 4, 2007
Published: December 3, 2007

REFERENCES

Anastasiou, E., and Lenhard, M. (2007). Growing up to one's standard. Curr. Opin. Plant Biol. *10*, 63–69.

Booker, J., Sieberer, T., Wright, W., Williamson, L., Willett, B., Stirnberg, P., Turnbull, C., Srinivasan, M., Goddard, P., and Leyser, O. (2005). MAX1 encodes a cytochrome P450 family member that acts downstream of MAX3/4 to produce a carotenoid-derived branch-inhibiting hormone. Dev. Cell *8*, 443–449.

Breitling, R., Armengaud, P., Amtmann, A., and Herzyk, P. (2004). Rank products: a simple, yet powerful, new method to detect differentially regulated genes in replicated microarray experiments. FEBS Lett. *573*, 83–92.

Cabello-Hurtado, F., Batard, Y., Salaün, J.P., Durst, F., Pinot, F., and Werck-Reichhart, D. (1998). Cloning, expression in yeast, and functional characterization of CYP81B1, a plant cytochrome P450 that catalyzes in-chain hydroxylation of fatty acids. J. Biol. Chem. *273*, 7260–7267.

Day, S.J., and Lawrence, P.A. (2000). Measuring dimensions: the regulation of size and shape. Development *127*, 2977–2987.

Deveaux, Y., Peaucelle, A., Roberts, G.R., Coen, E., Simon, R., Mizukami, Y., Traas, J., Murray, J.A., Doonan, J.H., and Laufs, P. (2003). The ethanol switch: a tool for tissue-specific gene induction during plant development. Plant J. *36*, 918–930.

Dharmasiri, N., Dharmasiri, S., Weijers, D., Lechner, E., Yamada, M., Hobbie, L., Ehrismann, J.S., Jurgens, G., and Estelle, M. (2005). Plant development is regulated by a family of auxin receptor F box proteins. Dev. Cell *9*, 109–119.

Dinneny, J.R., Yadegari, R., Fischer, R.L., Yanofsky, M.F., and Weigel, D. (2004). The role of JAGGED in shaping lateral organs. Development *131*, 1101–1110.

Dinneny, J.R., Weigel, D., and Yanofsky, M.F. (2006). NUBBIN and JAGGED define stamen and carpel shape in *Arabidopsis*. Development *133*, 1645–1655.

Disch, S., Anastasiou, E., Sharma, V.K., Laux, T., Fletcher, J.C., and Lenhard, M. (2006). The E3 ubiquitin ligase BIG BROTHER controls *Arabidopsis* organ size in a dosage-dependent manner. Curr. Biol. *16*, 272–279.

Donnelly, P.M., Bonetta, D., Tsukaya, H., Dengler, R.E., and Dengler, N.G. (1999). Cell cycling and cell enlargement in developing leaves of *Arabidopsis*. Dev. Biol. *215*, 407–419.

Duan, H., and Schuler, M.A. (2005). Differential expression and evolution of the *Arabidopsis* CYP86A subfamily. Plant Physiol. *137*, 1067–1081.

Gentleman, R.C., Carey, V.J., Bates, D.M., Bolstad, B., Dettling, M., Dudoit, S., Ellis, B., Gautier, L., Ge, Y., Gentry, J., et al. (2004). Bioconductor: open software development for computational biology and bioinformatics. Genome Biol. *5*, R80.

Groves, J.T. (2004). Models and mechanisms of cytochrome P450 action. In Cytochrome P450: Structure, Mechanism, and Biochemistry, Third edition, P.R. Ortiz de Montellano, ed. (New York: Springer), pp. 1–44.

Guzman, P., and Ecker, J.R. (1990). Exploiting the triple response of *Arabidopsis* to identify ethylene-related mutants. Plant Cell *2*, 513–523.

Hall, M.N. (2004). Cell Growth: Control of Cell Size (New York: Cold Spring Harbor Laboratory Press).

Haseloff, J., Siemering, K.R., Prasher, D.C., and Hodge, S. (1997). Removal of a cryptic intron and subcellular localization of green fluorescent protein are required to mark transgenic *Arabidopsis* plants brightly. Proc. Natl. Acad. Sci. USA *94*, 2122–2127.

Haubrick, L.L., and Assmann, S.M. (2006). Brassinosteroids and plant function: some clues, more puzzles. Plant Cell Environ. *29*, 446–457.

Horiguchi, G., Kim, G.T., and Tsukaya, H. (2005). The transcription factor AtGRF5 and the transcription coactivator AN3 regulate cell proliferation in leaf primordia of *Arabidopsis thaliana*. Plant J. *43*, 68–78.

Hu, Y., Xie, Q., and Chua, N.H. (2003). The *Arabidopsis* auxin-inducible gene ARGOS controls lateral organ size. Plant Cell *15*, 1951–1961.

Hu, Y., Poh, H.M., and Chua, N.H. (2006). The *Arabidopsis* ARGOS-LIKE gene regulates cell expansion during organ growth. Plant J. *47*, 1–9.

Hufnagel, L., Teleman, A.A., Rouault, H., Cohen, S.M., and Shraiman, B.I. (2007). On the mechanism of wing size determination in fly development. Proc. Natl. Acad. Sci. USA *104*, 3835–3840.

Imaishi, H., Matsuo, S., Swai, E., and Ohkawa, H. (2000). CYP78A1 preferentially expressed in developing inflorescences of *Zea mays* encoded a cytochrome P450-dependent lauric acid 12-monooxygenase. Biosci. Biotechnol. Biochem. *64*, 1696–1701.

Ingram, G.C., and Waites, R. (2006). Keeping it together: co-ordinating plant growth. Curr. Opin. Plant Biol. *9*, 12–20.

Ito, T., and Meyerowitz, E.M. (2000). Overexpression of a gene encoding a cytochrome P450, CYP78A9, induces large and seedless fruit in *Arabidopsis*. Plant Cell *12*, 1541–1550.

Jander, G., Norris, S.R., Rounsley, S.D., Bush, D.F., Levin, I.M., and Last, R.L. (2002). *Arabidopsis* map-based cloning in the post-genome era. Plant Physiol. *129*, 440–450.

Kim, G.T., Fujioka, S., Kozuka, T., Tax, F.E., Takatsuto, S., Yoshida, S., and Tsukaya, H. (2005). CYP90C1 and CYP90D1 are involved in different steps in the brassinosteroid biosynthesis pathway in *Arabidopsis thaliana*. Plant J. *41*, 710–721.

Kim, J.H., and Kende, H. (2004). A transcriptional coactivator, AtGIF1, is involved in regulating leaf growth and morphology in *Arabidopsis*. Proc. Natl. Acad. Sci. USA *101*, 13374–13379.

Li, J., and Chory, J. (1997). A putative leucine-rich repeat receptor kinase involved in brassinosteroid signal transduction. Cell *90*, 929–938.

Miyoshi, K., Ahn, B.O., Kawakatsu, T., Ito, Y., Itoh, J., Nagato, Y., and Kurata, N. (2004). PLASTOCHRON1, a timekeeper of leaf initiation in rice, encodes cytochrome P450. Proc. Natl. Acad. Sci. USA *101*, 875–880.

Mizukami, Y., and Fischer, R.L. (2000). Plant organ size control: AINTEGUMENTA regulates growth and cell numbers during organogenesis. Proc. Natl. Acad. Sci. USA *97*, 942–947.

Moore, I., Galweiler, L., Grosskopf, D., Schell, J., and Palme, K. (1998). A transcription activation system for regulated gene expression in transgenic plants. Proc. Natl. Acad. Sci. USA *95*, 376–381.

Nath, U., Crawford, B.C., Carpenter, R., and Coen, E. (2003). Genetic control of surface curvature. Science *299*, 1404–1407.

Nemhauser, J.L., Hong, F., and Chory, J. (2006). Different plant hormones regulate similar processes through largely nonoverlapping transcriptional responses. Cell *126*, 467–475.

Ohno, C.K., Reddy, G.V., Heisler, M.G., and Meyerowitz, E.M. (2004). The *Arabidopsis* JAGGED gene encodes a zinc finger protein that promotes leaf tissue development. Development *131*, 1111–1122.

Palatnik, J.F., Allen, E., Wu, X., Schommer, C., Schwab, R., Carrington, J.C., and Weigel, D. (2003). Control of leaf morphogenesis by microRNAs. Nature *425*, 257–263.

Peng, J., and Harberd, N.P. (1993). Derivative alleles of the *Arabidopsis* gibberellin-insensitive (gai) mutation confer a wild-type phenotype. Plant Cell *5*, 351–360.

Pinot, F., Skrabs, M., Compagnon, V., Salaun, J.P., Benveniste, I., Schreiber, L., and Durst, F. (2000). ω-Hydroxylation of epoxy- and hydroxy-fatty acids by CYP94A1: possible involvement in plant defence. Biochem. Soc. Trans. *28*, 867–870.

Riefler, M., Novak, O., Strnad, M., and Schmulling, T. (2006). *Arabidopsis* cytokinin receptor mutants reveal functions in shoot growth, leaf senescence, seed size, germination, root development, and cytokinin metabolism. Plant Cell *18*, 40–54.

Saeed, A.I., Bhagabati, N.K., Braisted, J.C., Liang, W., Sharov, V., Howe, E.A., Li, J., Thiagarajan, M., White, J.A., and Quackenbush, J. (2006). TM4 microarray software suite. Methods Enzymol. *411*, 134–193.

Sakakibara, H. (2006). Cytokinins: activity, biosynthesis, and translocation. Annu. Rev. Plant Biol. *57*, 431–449.

Schuler, M.A., and Werck-Reichhart, D. (2003). Functional genomics of P450s. Annu. Rev. Plant Biol. *54*, 629–667.

Shpak, E.D., Berthiaume, C.T., Hill, E.J., and Torii, K.U. (2004). Synergistic interaction of three ERECTA-family receptor-like kinases controls *Arabidopsis* organ growth and flower development by promoting cell proliferation. Development *131*, 1491–1501.

Soukas, A., Cohen, P., Socci, N.D., and Friedman, J.M. (2000). Leptin-specific patterns of gene expression in white adipose tissue. Genes Dev. *14*, 963–980.

Sugimoto-Shirasu, K., Roberts, G.R., Stacey, N.J., McCann, M.C., Maxwell, A., and Roberts, K. (2005). RHL1 is an essential component of the plant DNA topoisomerase VI complex and is required for ploidy-dependent cell growth. Proc. Natl. Acad. Sci. USA *102*, 18736–18741.

Szecsi, J., Joly, C., Bordji, K., Varaud, E., Cock, J.M., Dumas, C., and Bendahmane, M. (2006). BIGPETALp, a bHLH transcription factor is involved in the control of *Arabidopsis* petal size. EMBO J. *25*, 3912–3920.

Tamaki, K., Imaishi, H., Ohkawa, H., Oono, K., and Sugimoto, M. (2005). Cloning, expression in yeast, and functional characterization of CYP76A4, a novel cytochrome P450 of petunia that catalyzes (ω-1)-hydroxylation of lauric acid. Biosci. Biotechnol. Biochem. *69*, 406–409.

Teale, W.D., Paponov, I.A., and Palme, K. (2006). Auxin in action: signalling, transport and the control of plant growth and development. Nat. Rev. Mol. Cell Biol. *7*, 847–859.

Tilly, J.J., Allen, D.W., and Jack, T. (1998). The CArG boxes in the promoter of the *Arabidopsis* floral organ identity gene APETALA3 mediate diverse regulatory effects. Development *125*, 1647–1657.

Tissier, A.F., Marillonnet, S., Klimyuk, V., Patel, K., Torres, M.A., Murphy, G., and Jones, J.D. (1999). Multiple independent defective suppressor-mutator transposon insertions in *Arabidopsis*: a tool for functional genomics. Plant Cell *11*, 1841–1852.

Weigel, D., Ahn, J.H., Blazquez, M.A., Borevitz, J.O., Christensen, S.K., Fankhauser, C., Ferrandiz, C., Kardailsky, I., Malancharuvil,

E.J., Neff, M.M., et al. (2000). Activation tagging in *Arabidopsis*. Plant Physiol. *122*, 1003–1013.

White, D.W. (2006). PEAPOD regulates lamina size and curvature in *Arabidopsis*. Proc. Natl. Acad. Sci. USA *103*, 13238–13243.

Yeung, K.Y., Haynor, D.R., and Ruzzo, W.L. (2001). Validating clustering for gene expression data. Bioinformatics *17*, 309–318.

Zondlo, S.C., and Irish, V.F. (1999). CYP78A5 encodes a cytochrome P450 that marks the shoot apical meristem boundary in *Arabidopsis*. Plant J. *19*, 259–268.

Accession Numbers

The microarray data have been submitted to the Gene Expression Omnibus (GEO) repository (http://www.ncbi.nlm.nih.gov/geo) with accession number GSE9201.